

## Journal of Animal and Poultry Production

Journal homepage: [www.japp.mans.edu.eg](http://www.japp.mans.edu.eg)  
Available online at: [www.jappmu.journals.ekb.eg](http://www.jappmu.journals.ekb.eg)

### Identification of Sarcocystis Species "Macrocytis" by Visual and Molecular Technique in Sheep and Goats -Sulaymaniyah Slaughterhouse.



Shabang Mahmood Salam\* and Bahzad Hama Salih Mustafa

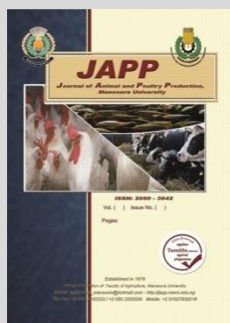
Cross Mark

Animal Science Department-College of Agricultural Engineering Sciences-University of Sulaymaniyah

#### ABSTRACT

This study aimed to investigate factors established to be independently associated with parasitic infection such as the gender, seasons and tissues (oesophagus, heart, diaphragm and tongue). Examination of 194897 sheep out of which 129635 males and 65262 females revealed that 9062 sheep were found to be infected (6128 males and 2934 females). The examination of 27720 goats out of which 17809 males and 9911 females exposed those 969 goats were found to be infected (491 males and 478 females). Also, results showed that incidences of sheep sarcocystosis among the studied sheep observed to be gradually decrease from the highest infection percent of 5.5% in summer, 4.7% in Autumn, 4.4% in winter to reach its minimum during spring (4%). Regarding goats sarcocystosis among the studied goats observed to be gradually decrease from the highest infection percent of 3.5% in summer, 3.4% in Autumn, 3.4% in winter to reach its minimum during spring (2.7%). For sheep regarding Mincing and tissue squash method, 62% of sheep were infected in esophagus (48% males and 14% females), 52% were infected in heart (42% males and 10% females), 24% were infected in diaphragm (18% males and 6% females) and 68% were infected in tongue (52% males and 16% females). Meanwhile in Cellophane adhesive, 48% of sheep were infected in esophagus (36% males and 12% females), 50% were infected in heart (38% males and 12% females), 26% were infected in diaphragm (18% males and 8% females) and 72% were infected in tongue (50% males and 22% females).

**Keywords:** *Sarcocystis* spp. (Macrocytis), Sheep, Goats, Sulaymaniyah Slaughterhouse



#### INTRODUCTION

Sarcocystis was first reported in 1843 by Miescher as white threadlike cysts in striated muscles of a house mouse, without a scientific name. Then after 20 years, the parasite was simply referred to as Meischer's tubules. Sarcocystis spp. are cyst forming intracellular protozoan parasites with an obligatory prey predator. Two host lifecycle. Asexual stages develop in intermediate hosts after they ingest the oocyst stage from definitive-host faeces and terminate with the formation of intramuscular cysts Sarcocystis (Jehle *et al.*, 2009).

Sarcocystosis identified as one of the most commonly protozoan diseases in the world, it has specific life cycle, because it depends on prey – predator relationship. Carnivores acting as definitive, as well as humans, while the intermediate host are mainly livestock animals, which their meat are consumed by the final hosts, include cattle, buffalo, sheep, and goats, as well as pigs (Şaki *et al.*, 2010). These animals affected with asexual form of parasite which called (Tissue or muscular cyst), studies registered that frozen buffalo meat has multiple Sarcocystis infections (Mohamed *et al.*, 2016; Elkady *et al.*, 2018). Typically, the definitive hosts do not show any clinical signs of Sarcocystosis, while the disease mainly was asymptomatic in intermediate host when infected. Occasionally, some animals have clear clinical sings according the site of tissue cyst such as mild fever, diarrhea, chills, general weakness, respiratory problems may occur and neurological sings (Faraj and Kawan, 2012; Lau *et al.*, 2014). Traditional methods such as macroscopic and microscopic techniques are mainly used to diagnosis,

additionally, molecular assay are a range of DNA based technique for the detection of Sarcocystis parasites (Kawan, 2019). Sarcocystosis infections in small ruminants such as sheep and goats are common and are the major cause of abortions worldwide resulting in major economic losses in sheep and goat industries (Buxton *et al.*, 2007; Dubey, 2010; Innes, 2010; Innes *et al.*, 2009). Sheep can also be infected with several different species of Sarcocystis, and which are normally identified during microscopic examination. Carcasses showing macroscopic cysts are normally discarded during meat inspections causing major production losses and the cost of Sarcocystis infections in small ruminant was estimated to be around 20 million euros a year in a study in Spain from 145 farms (Martínez-Navalón *et al.*, 2012). Among the previously reported species of Sarcocystis, *S. tenella* and *S. arietianis* (transmitted by canids) are frequently identifiable by microscopic cysts morphology, and *S. gigantean* and *S. medusiformis* (transmitted by cats) are identified by the observation of macroscopic cysts in the muscle tissue (Dubey *et al.*, 2015; Ortega-Mora, 2007). The aims of this study are knowledge of the prevalence and identification of Sarcocystosis (micro and macro cysts in different site and organs) among sheep and goats and estimate the molecular prevalence and genetic characterization of Sarcocystis species in Sulaymaniyah slaughterhouse.

#### MATERIALS AND METHODS

During the course of this study which extended from August 2020 to January 2021, tissue samples involving the

\* Corresponding author.

E-mail address: [shabang.010017@univsul.edu.iq](mailto:shabang.010017@univsul.edu.iq)

DOI: 10.21608/jappmu.2021.204699

esophagus, diaphragm and heart were randomly collected from 200 sheep (male and female) and 200 goats (male and female) in general slaughterhouse in Sulaymaniyah province, careful examination was conducted prior to slaughtering to make sure that all included sheep and goats were healthy before being slaughtered.

**Samples collection**

Tissue samples involving the esophagus, diaphragm and heart were randomly collected from 200 sheep (male and female) and 200 goats (male and female) in slaughterhouse. Different samples were collected also different seasons from different organs, isolated, *Macrocyctis* spp., in diaphragm, esophagus and heart in sheep and goats.

**Molecular study:**

The number of samples taken is 80, which 40 samples from sheep and 40 samples goats. Also, 20 of esophagus and 20 from diaphragm. For molecular analysis, soft cysts of the macrocystis were dissected, washed several times in 0.01 M phosphate-buffered saline (pH 7.2), and stored at -20 °C until DNA extraction.

**DNA extraction:**

G-spin™ Total DNA Extraction Mini Kit, Korea was used for DNA extraction where according to provider’s protocol and after collecting extracted macrocystis in a Petri dish. 20mg of tissue and transferred into a 1.5 ml micro-centrifuge tube and added 200 µl of Lysis Solution. Added 20 µl of proteinase K solution (20 mg/ml) to the sample tube, mixed by vortexing, and incubated at 56 °C until the tissue was completely lysed: Vortexed occasionally during incubation to disperse the sample for 15 minutes, then added 200 µl of Binding Solution to the sample tube, and mixed

well by pulse-vortexing for 15 sec. Incubated at 56 °C for 10 min. Added 200 µl of absolute ethanol and mixed well by pulse-vortexing for 15 sec. after this step, briefly spun down to get the drops cleaning under the lid, carefully transferred the lysate into the upper reservoir of the spin column with 2.0 ml collection tube without wetting the rim. Centrifuged at 13000 rpm for 1 min: Pour off the flow-through and assemble the spun column with the 2.0 ml collection tube. Added 500 µl of Washing 1 solution to the spin column with collection tube and centrifuged at 13,000 rpm for 1 min: pour off the flow-through and assemble the spin column with the 2.0 ml collection tube. Added 500 µl of washing 2 solution to the spin column with collection tube and centrifuged at 13,000 rpm for 1 min: pour off the flow-through and assemble the spin column with the 2.0 ml collection tube. Dried the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column, after transferred the spin column to the new 1.5 ml micro-centrifuge tube. Added 150 µl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 min. Eluted the genomic DNA by centrifugation at 13,000 rpm for 1 min.

**PCR-reaction:**

PCR reaction was carried out in 30 µl of Premix (AccuPower PCR PreMix, Korea). Twenty-five microliters of Taq Master Mix were used with 10 ng template DNA, 0.1 µM of each primer and distilled water. Cycles of PCR are summarized in (Table- 1). the amplified DNA obtained after PCR procedure was electrophoresed using 2% agarose gel, stained with Ethidium Bromide (0.5 µg/ml) and visualized under the UV light.

**Table 1. Summary of steps involved in PCR.**

Primer	Amplification program			
	Stage	Temperature (C°)	Time	cycles
Forward (F): 5’GCACTTGATGAATTCTGGCA3’	Denaturation	95	5 min	35X
Reverse (R): 5’CACCACCCATAGAATCAAG 3’	Denaturation	95	30 sec	
Product: 600 Bp (Bahari, <i>et al.</i> ,2014)	Annealing	55	45 sec	
2LF: GGATAAACCGTGGTAATTCTATG	Extension	72	30 sec	
Primer 3HR: GGCAAATGCTTTCGCAGTAG				
Product: 850 Bp Choi <i>et al.</i> (2018); Kalantari <i>et al.</i> (2016); Yang <i>et al.</i> (2001); Calero-Bernal <i>et al.</i> (2014)				
Primer S1F: GAATCCAAACCCCTTTCAGAGT	Final	72	10 in	
Primer 1HR: TATCCCATCACGATGCATAC				
Product: 1050 Bp (Choi <i>et al.</i> (2018); Formisano <i>et al.</i> (2013)				

**Statistically analysis**

Statistical analysis was done to evaluate the results with a confidence interval using Chi-square test independence (p < 0.01) between the seasons, organs (Esophagus, Diaphragm and Heart) and sex in sheep and goats.

**RESULTS AND DISCUSSION**

**Results**

The prevalence of the revealed sarcocystis were reported in all seasons of the year in Sulaymaniyah slaughterhouse during different seasons in 2020-2021. Our results as illustrated in Table (2), showed the highly significant between seasons that incidences of sheep sarcocystosis among the studied sheep observed to be gradually decrease from the highest infection percent of 5.5% in summer, 4.7% in Autumn, and 4.4% in winter to

reach its minimum during spring (4%). Regarding males, the highest infection rate was in autumn (5.84%) while the lowest was spring (3.09%). On the other hand, the highest infection rate in females was in spring (5.89%) while the lowest was in Autumn (2.56%).

Our results as illustrated the highly significant between seasons in Table (3), showed that incidences of goat’s sarcocystosis among the studied goats observed to be gradually decrease from the highest infection percent of 3.8% in summer, 3.6% in autumn and winter to reach its minimum during spring (2.9%). Regarding males, the highest infection rate was in summer (3.37%) while the lowest was spring (2.17%). On the other hand, the highest infection rate in females was in winter (5.89%) while the lowest was in spring (4.35%). The infection rate found to be higher in females than males.

**Table 2. The prevalence rate of *Macrocystis* spp., in sheep in Sulaymaniyah slaughter house during different seasons in 2020-2021.**

Seasons	No. of examination	No. of infections %	Male	+/%	-/%	Female	+/%	-/%
Summer	48711	2688 5.5%	32400	1779 5.49%	30621 94.51%	16311	909 5.57%	15402 94.43%
Autumn	47576	2257 4.7%	31644	1849 5.84%	29795 94.16%	15932	408 2.56%	15524 97.45%
Winter	43235	1886 4.4%	28758	1361 4.73%	27397 95.27%	14477	525 3.63%	13952 96.37%
Spring	55374	2231 4.0%	36832	1139 3.09%	35693 96.91%	18542	1092 5.89%	17450 94.11%
Total	194896	9062	129634	6128	123506	65262	2934	62328

**Table 3. The prevalence rate of *Macrocystis* spp., in goats in Sulaymaniyah slaughter house during different seasons in 2020-2021.**

Seasons	No. of Examination	No. of infections %	Male	+%	-%	Female	+%	-%
Summer	8364	317 3.8%	5374	181 3.37%	5193 96.63%	2990	136 4.55%	2854 95.45%
Autumn	6560	238 3.6%	4215	118 2.8%	4097 97.2%	2345	120 5.12%	2225 94.88%
Winter	5396	196 3.6%	3466	89 2.57%	3377 97.43%	1930	107 5.54%	1823 94.46%
Spring	7400	218 2.9%	4754	103 2.17%	4651 97.83%	2646	115 4.35%	2531 95.65%
Total	27720	969	17809	491	17318	9911	478	9433

Examination of 194897 sheep out of which 129635 males and 65262 females as presented in Table (4) revealed that 9062 sheep were found to be infected (6128 males and 2934 females) resemble 4.7% of the total examined sheep. Out of the infected sheep were (67.6% males and 32.4% females). Examination of 27720 goats out of which 17809

males and 9911 females as presented in Table (4) revealed that 969 goats were found to be infected (491 males and 478 females) resemble 3.5 % of the total examined goats. Our results is highly significant between prevalence sheep and goats because P value <0.01, Out of the infected goats 50.7% were males and 49.3% females.

**Table 4. The prevalence rate of *Sarcocystosis* (*Macrocystis*) in sheep and goats in 2020-2021.**

Animals	No. of examined	No. of infected %	Male	+/%	-/%	Female	+/%	-/%
Sheep	194897	9062 4.7%	129635	6128 67.6%	123507 95.27%	65262	2934 32.4%	62328 95.5%
Goats	27720	969 3.5%	17809	491 50.7%	17318 97.24%	9911	478 49.3%	9433 95.18%
Total	222617	10031	147444	6619	140825	75173	3412	71761

In the sheep samples screened 9062 sheep were found to be infected including 6146 males (67.8%) and 2916 females (32.2%),(Table 5). In this result shows the highly significant between the different organs, regarding male sheep, the highest positive rate which was 80.4% occurred in male diaphragm, followed by esophagus was 59.9% and 57% of heart, the highly positive prevalence cases of female shows in heart 43.0%, followed the esophagus 40.1%, and the lowest in of diaphragm19.6%.

**Table 5. The prevalence rate of *Macrocystis* spp., in different organs in goats during 2020-2021.**

Tissues/ organs	No. Positive	Male	%	Female	%
Esophagus	5414	3242	59.9	2172	40.1
Heart	128	73	57	55	43
Diaphragm	3520	2831	80.4	689	19.6
Total	9062	6146	67.8	2916	32.2

In the goat samples screened 969 goats were found to be infected including 491 males (50.7%) and 478 females (49.3%). (Table 6).Our result shows the highly significant, regarding male goats, the highest positive rate which was 67.3% occurred in male diaphragm, followed by heart 54.0% and esophagus 41.5%. On the other hand, regarding the positive prevalence cases of female goats the highest positive rate which was 58.5% occurred in esophagus, followed by heart 46% and diaphragm 32.7% the lowest. .

**Table 6. The prevalence rate of *Macrocystis* in goats in Sulaymaniyah slaughter house in different organs during 2020-2021.**

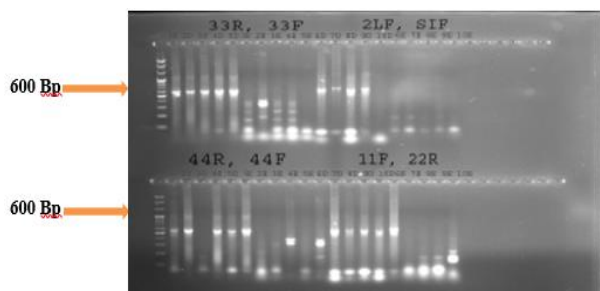
Tissues/ organs	No. positive	Male	%	Female	%
Esophagus	561	233	41.5	328	58.5
Heart	124	67	54	57	46
Diaphragm	284	191	67.3	93	32.7
Total	969	491	50.7	478	49.3

Identification of sarcocystis species in different organs in sheep, using a molecular test.

**Molecular characterization of samples isolated from sheep:**

The PCR amplification revealed that the 20 isolated samples from diaphragm and esophagus of sheep where 5 compared to 33R, 33F primer, 5 to 2LF, SIF primer, 5 to 44R, 44F primer and 5 to 11F, 22R primer while 20 samples from diaphragm of sheep where 5 compared to 33R, 33F primer, 5 to 2LF, SIF primer, 5 to 44R, 44F primer and 5 to 11F, 22R primer.

The partial 18S rRNA gene was amplified in all tested samples and yielded the expected amplicon PCR size of 600 base pairs for the 18 out of the 20 examined diaphragm and samples while showed no bands clear bands for 16 samples of esophagus while three samples showed band in comparison with 44R, 44F and 33R, 33F primers around 500 base pair and one showed a band in comparison with 11F, 22R primer at and yielded the expected amplicon PCR with molecular size of 600 base pairs the expected 600 base pair as illustrated in figure 1.



**Figure 1.** PCR products of partial 18S rRNA gene of *Sarcocystis* spp. isolated from sheep, presented bands at 600 base pair on 1% agarose gel. M: 100 bp size marker. 1-10 E (samples isolated from esophagus) and 1-10 D (samples isolated from diaphragm).

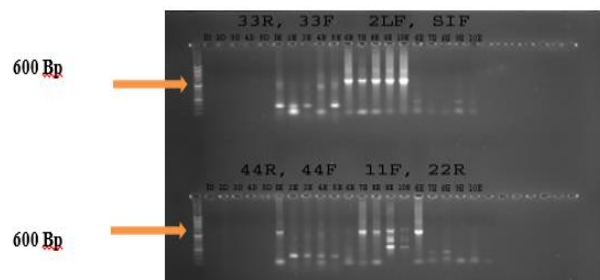
The PCR amplification revealed that 40 isolates produced a positive band on gel electrophoresis with different amplicon molecular sizes. The partial rRNA gene was amplified in all tested samples and yielded the expected amplicon PCR size of 850, 690, 600 and 570 bp in sheep.

Identification of sarcocysts species in different organs in goats, using a molecular test.

**Molecular characterization of samples isolated from goats:**

The PCR amplification revealed that the 20 isolated samples from diaphragm of goats where 5 compared to 33R, 33F primer, 5 to 2LF, SIF primer, 5 to 44R, 44F primer and 5 to 11F, 22R primer while 20 samples from diaphragm of goats where 5 compared to 33R, 33F primer, 5 to 2LF, SIF primer, 5 to 44R, 44F primer and 5 to 11F, 22R primer. The samples from diaphragm of goats showed no bands in comparison with 33R, 33F primer and showed clear bands with isolates produced a positive band on gel electrophoresis and yielded the expected amplicon PCR with molecular size of 600 base pairs for the 5 samples compared 2LF, SIF primer samples while showed no bands for the 5 samples compared with 44R, 44F primer and also showed clear bands with the 5 samples compared with 11F, 22R primer and yielded the expected amplicon PCR with molecular size of 600 base pairs as illustrated in figure 2.

Regarding the 20 samples of esophagus none of the samples yielded clear band in comparison with the used primers except only one band showed the expected band around 600 base pair in comparison with 44R, 44F primer as illustrated in figure 2.



**Figure 2.** PCR products of partial 18S rRNA gene of *Sarcocystis* spp. isolated from goats, presented bands at 600 base pair on 1% agarose gel. M: 100 bp size marker. 1-10 E (samples isolated from esophagus) and 1-10 D (samples isolated from diaphragm).

The PCR amplification revealed that 40 isolates produced a positive band on gel electrophoresis with different amplicon molecular sizes. The partial rRNA gene was amplified in all tested samples and yielded the expected amplicon PCR size of, 850, 690, 600 and 570 bp in goats.

This is the molecular and ultrastructural study of ovine and caprine sarcocystis infection in Sulaymaniyah Kurdistan, Iraq. The present finding showed that the isolated sarcocystis spp. Were most closely related to *S. gigantea*, *S. moulei*, and *S. medusiformis* and may consider them as sibling strains; the cross-infection may happen among sheep and goats.

**Discussion**

Our results as illustrated in Table (2), showed the highly significant seasons that incidences of sheep sarcocystosis among the studied sheep observed to be gradually decrease from the highest infection percent of 5.5% in summer and 4.7% in Autumn, our result closely similar with (Mahran, 2009) and (Aly, 2012) who stated that sarcocysts infection in sheep reaches its peak during Winter and Summer, respectively. A hypothesis might explain the variation in the results of the previously mentioned studies is that, the cysts represent the chronic, longest and persistent stage of *Sarcocystis* species life cycle (Beyer and Radchenko, 2001). Temperature has an important role on the viability of *Sarcocystis* sporocysts in the pasture (Dubey *et al.* 1989). Prevalence of explained by the assumption that during the hot and therefore, once the cyst is formed, it persists in the tissues for long period and could be noticed all over the year. Prevalence rates of *Sarcocystis* infections in sheep worldwide can vary between different countries, and can range from an undetectable level to 100%, showing that the genus *Sarcocystis* is widespread in various countries (Dubey *et al.*, 2015).

The results as illustrated the highly significant between seasons in Table (3), showed that incidences of goat's sarcocystosis among the studied goats observed to be gradually decrease from the highest infection percent of 3.8% in summer, High prevalence of *Sarcocystis* microscopic and macroscopic cysts have been recorded in goats (Dubey *et al.*, 2015) which disagreement with our results. In a study in Riyadh, in Saudi Arabia, 77% of goats slaughtered for meat consumption were shown to have natural infections with *Sarcocystis* species (Al-Hoot *et al.*, 2005) and in the study by (Amairia *et al.*, 2018) infections with *Sarcocystis* were reported to be 50.4% from 121 slaughtered goats from a regional slaughterhouse in north-west Tunisia. Moreover, a study in south-western China, 77.3% of goats infected with sarcocystis (Hu *et al.*, 2016) The epidemiological study of *Sarcocystis* infection in goats was 71% in Bangalore. The results of the survey were in agreement with (Lal Singh, 1991), (Dayashanker, 1991) and (Agarwal *et al.*, 1991) who reported higher prevalence rates. But (Aulakh, 1997) and (Wadajkar, 1994) reported lower prevalence rate in Ludhiana and Marathwada regions, respectively.

The results presented in Table (4), the highest infection in sheep males is (67.6%) and infected goats were 3.5 % which lowest rate in females which (49.3%). that our study closely agreement with (Al Quraishy *et al.*, 2014) in the study in Saudi Arabia, sarcocystis spp. were identified in 85.8% of domestic sheep. High prevalence of *Sarcocystis*

microscopic and macroscopic cysts have been recorded in goats (Dubey *et al.*, 2015). In Duhok province (Hussein, 2015) found the rate 1.2% and 2.6% in sheep and goats respectively.

In (Table 5 and 6) the results show the highly significant between the different organs, regarding, the highest positive rate in esophagus, followed by diaphragm and lowest rate of heart in sheep and goats respectively, our result agreement with Beyazit *et al.*, (2007) noted the highest prevalence of macrocysts in the esophagus in a group of sheep and goats studied in Turkey, and also (Barham *et al.*, 2005) found the highest rate of infection 99% in esophagus and lowest rate 3% in diaphragm of goats in Sulaymaniyah province.

The distribution of sarcocysts in different tissues of goats was studied by (Lal Singh, 1991) and (Agarwal *et al.*, 1991) where in esophagus the most was infected among all other tissues screened. However, (Dayashanker, 1991) reported diaphragm as the most infected tissue followed by esophagus, while our result agreement with (Singh *et al.*, 1990) reported tail muscles as primary site of infection. In the present study also, esophagus was the most infected organ in the female goats.

In our results of ultrastructural and molecular analysis of macroscopic sarcocysts revealed three different types of sarcocystis in diaphragm and esophagus of (sheep and goats), the results indicated that all of them represented *S. gigantea*, *S. medusiformis* and *S. moulei*. There are only two validated macroscopic species of *Sarcocystis* described in sheep; *S. gigantea* and *S. medusiformis*. *S. gigantea* spread all over the world, including Iraq (Al-Hyali *et al.*, 2011), whereas *S. medusiformis* was only reported in Italy, Iran, New Zealand, Spain, Jordan and Australia (Collins *et al.*, 1979; Dubey *et al.*, 2016). The presence of *S. moulei* in the ovine esophagus has indicated that sheep may be a suitable host for *S. moulei* which has previously been documented for goat parasitization. Our result is agreement with Elmishmishy *et al.* (2018) stated that a very close phylogenetic interaction occurred between *S. gigantea* and *S. moulei*, a specific goat species rarely recorded in sheep, and suggested cross-transmission between these two hosts. Furthermore, similar results were recorded in Iran and suggest that sheep can be a convenient and alternative host for *S. moulei*. (Kalantari *et al.*, 2016). Furthermore, other researchers concluded that *Sarcocystis* spp. sheep and goats are closely related and should be grouped together (Metwally *et al.*, 2019).

In this study, molecular detection was performed using partial 18S rRNA gene for confirmation of *Sarcocystis* spp. genotype. Variable regions of the 18S rRNA gene serve as useful targets for the classification and characterization of different species (Neefs *et al.*, 1991; Yang *et al.*, 2001). Furthermore, 18S rDNA is particularly suitable for phylogenetic investigations as its high conservation and examination of its variable regions allows identification of species within the genus. (Maidak *et al.*, 1997; Ng *et al.*, 2015). Generally, *S. gigantea* and *S. medusiformis* has been reported less frequently compared to *S. tenella* and *S. arieticanis* (Dubey *et al.*, 2015; Gual *et al.*, 2017) which is close to our result. A study in Brazil examined tissue for *Sarcocystis* spp., by light and electron microscopy, macroscopic evaluation and molecular tests and detected

microscopic cysts of *Sarcocystis* spp. in 91.6 % of goat sample tested (Bittencourt *et al.*, 2016). The prevalence of *S. moulei* seems to be relatively low in comparison to the other *Sarcocystis* species identified in goats.

## CONCLUSION

The results of this study showed a high frequency of *Sarcocystis* species infection in sheep and goats slaughtered in the Sulaymaniyah slaughterhouse. There is a highly significant relationship between the occurrence of infection and the season. The diaphragm and esophagus are the most infected organ among sheep and goats, but lowest infection in heart, molecular and ultrastructural study of sheep and goats sarcocystis species in Sulaymaniyah, were most closely associated with *S. gigantea*, *S. moulei*, and *S. medusiformis* might and should and will contemplate them as relative strains; the cross-infection may happen among sheep and goats. it's of nice importance the farmers to be trained to not feed their dogs and cats with raw meat, and therefore the edifice remnants to be burned, so as to be effectively broken of infection cycle between the intermediate and therefore the definitive hosts. So, we have a tendency to suggested to require attention on abattoirs to stop transmission cycle of sarcosporidiosis.

## REFERENCES

- Agarwal, M.C., Singh, K.P. and Shah, H.L. (1991) Caprine sarcocystosis in Jabalpur area. *J. Vet. Parasitol.*, 5:108-112.
- Al-Hoot, A. S., Al-Qureishy, S. A., Al-Rashid, K. and Bashtar, A. R. (2005). Microscopic study on *Sarcocystis moulei* from sheep and goats in Saudi Arabia. *Journal of the Egyptian Society of Parasitology*, 35, 295-312.
- Al-Hyali NS, Kennany ER, and Khalil LY (2011). Fate of macrosarcocyst of *Sarcocystis gigantea* in sheep. *Iraqi Journal of Veterinary Sciences*, 25(2): 87-91. Available at: <http://www.vetmedmosul.org/ijvs>
- Aly, A. A. (2012): Prevalence of some Parasitic Infestation in Tissues of Animals Slaughtered in Qena Abattoirs, Egypt. Ph. D. Thesis Fac. Vet. Med. Assuit Univ.
- Al Quraishy, S., Morsy, K., Bashtar, A. R., Ghaffar, F. A. and Mehlhorn, H. (2014). *Sarcocystis arieticanis* (Apicomplexa: Sarcocystidae) infecting the heart muscles of the domestic sheep, *Ovis aries* (Artiodactyla: Bovidae), from K. S. A. on the basis of light and electron microscopic data. *Parasitology Research*, 113, 3823-3831.
- Amairia, S., Amdouni, Y., Rouatbi, M., Rjeibi, M. R., Awadi, S. and Gharbi, M. (2018). First detection and molecular identification of *Sarcocystis* spp. in small ruminants in North-West Tunisia. *Transboundary and Emerging Diseases*, 65, 441-446.
- Aulakh, R. S., Joshi, D. V. and Juyal, P. D. (1997): *J. Vet. Parasitol*, 11: 99-100.
- Barham, M., Stützer, H., Karanis, P., Latif, B. M., & Neiss, W. F. (2005). Seasonal variation in *Sarcocystis* species infections in goats in northern Iraq. *Parasitology*, 130(2), 151-156.
- Beyazit, A., Yazicioğlu, Ö., & Karaer, Z. (2007). The prevalence of ovine *Sarcocystis* species in Izmir province. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 54(2), 111-116.

- Beyer, T. V. & Radchenko, A. I. (2001): Intracellular Parasitism and the Problem of Sarcocystosis. *Biology Bulletin of the Russian Academy of Sciences*, 28(2): 119 - 125.
- Bittencourt, M. V., Meneses, I. D., Ribeiro-Andrade, M., de Jesus, R. F., de Araujo, F. R. and Gondim, L. F. (2016). *Sarcocystis* spp. in sheep and goats: frequency of infection and species identification by morphological, ultrastructural, and molecular tests in Bahia, Brazil. *Parasitology Research*, 115, 1683-1689.
- Buxton, D., Maley, S. W., Thomson, K. M., Trees, A. J. and Innes, E. A. (1997). Experimental infection of non-pregnant and pregnant sheep with *Neospora caninum*. *Journal of Comparative Pathology*, 117, 1-16.
- Collins GH, Atkinson E, and Charleston WAG (1979). Studies on *Sarcocystis* species III: the macrocystic species of sheep. *New Zealand Veterinary Journal*, 27(10): 204–206. DOI: <https://doi.org/10.1080/00480169.1979.34651>
- Dafedar, A. M., D'Souza, P. E., & Puttalakshamma, G. C. (2008). Prevalence of sarcocystosis in goats slaughtered at an abattoir in Bangalore, Karnataka state. *Veterinary World*, 1(11), 335-337.
- Dayashanker. (1991): Studies on epidemiological aspects of sarcocystis infection in domestic goat (*Capra hircus*) in Tarai (U.P) and its serodiagnosis with a reference to eimerian infection. M.V.Sc. thesis submitted to G. B. Pant University of Agriculture and Technology, Pantnagar.
- Dubey, J. P. (2010). *Toxoplasmosis of Animals and Humans* (Second Edition). *Parasites & Vectors*, 3, 112-112.
- Dubey, J. P., Calero-Bernal, R., Rosenthal, B. M., Speer, A. and Fayer, R. (2015). *Sarcocystosis of Animals and Humans*. Second Edition. *CRC Press, Taylor & Francis Group, Boca Raton, London, New York*.
- Dubey JP, Calero Bernal R, Rosenthal BM, Speer CA, Fayer. *Sarcocystosis of animals and humans*. 2nd Edn. Boca Raton: CRC Press; Taylor & Francis Group; 2016.
- Dubey, J. P.; Speer, C. A. and Fayer, R. (1989): *Sarcocystosis of animals and man*. CRC Press Inc, Boca Raton, Florida.
- El-kady, A.; Hussein, N. and Hassan, A. (2018). First molecular characterization of *Sarcocystis* spp. in cattle in Qena Governorate, Upper Egypt. *J Parasit Dis.*, 42: 114–121.
- Elmishmishy B, Al-Araby M, Abbas I, and Abu-Elwafa S (2018). Genetic variability within isolates of *Sarcocystis* species infecting sheep from Egypt. *Veterinary Parasitology: Regional Studies and Reports*, 13: 193–197. DOI: <https://doi.org/10.1016/j.vprsr.2018.07.002>
- Faraj, A.A. and Kawan, M.H. (2012). Detection of *Sarcocystosis* in some wild birds. *Iraqi J. Vet. Med.*, 36: 56-70.
- Gual, I., Bartley, P. M., Katzer, F., Innes, E. A., Canton, G. J. and Moore, D. P. (2017). Molecular confirmation of *Sarcocystis gigantea* in a naturally infected sheep in Argentina: A case report. *Veterinary Parasitology*, 248, 25-27.
- Hu, J. J., Liu, T. T., Liu, Q., Esch, G. W., Chen, J. Q., Huang, S., et al. (2016). Prevalence, morphology, and molecular characteristics of *Sarcocystis* spp. in domestic goats (*Capra hircus*) from Kunming, China. *Parasitology Research*, 115, 3973-3981.
- Hussein, S.H.N., 2015. Prevalence of *Sarcocystis* Infection in Small Ruminants (Sheep and Goats) and Experimental Infection in Dogs and Cats in Duhok Province. MSc Thesis Submitted to Council of College of Veterinary Medicine, University of Duhok.
- Innes, E. A. (2010). A brief history and overview of *Toxoplasma gondii*. *Zoonoses and Public Health*, 57, 1-7.
- Innes, E. A., Bartley, P. M., Buxton, D. and Katzer, F. (2009). Ovine toxoplasmosis. *Parasitology*, 136, 1887-1894.
- Jehle, C., A. Dinkel, A. Sander, M. Morent, T. Romig, P.V. Luc, T.V. De, V.V. Thai and U. Mackenstedt, (2009). Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. *Veterinary Parasitology*, 166: 314-320.
- Kalantari N, Khaksar M, Ghaffari S, and Hamidekish SM (2016). Molecular Analysis of *Sarcocystis* Spp. Isolated from Sheep (*Ovis aries*) in Babol area, Mazandaran Province, Northern Iran. *Iran Journal of Parasitology*, 11(1): 73–80. Available at: <https://pubmed.ncbi.nlm.nih.gov/27095971/>
- Kawan, M.H. (2019). Molecular surveillance and phylogenetic analysis of *Theileria annulata* in bovine at Baghdad city, Iraq *J Vet Med.*, 43: 93-101.
- Lal Singh, (1991). Studies on *Sarcocystis capracanis*. M.V.Sc. Thesis submitted to Rajasthan Agricultural University, Bikaner.
- Lau, Y.; Change, P.; Tan, C.; Fong, M.; Mahmud, R. and Wong, K. (2014). *Sarcocystis nesbitti* infection in human skeletal muscle: possible transmission from snakes. *Am J Trop Med Hyg.*, 90: 361–364.
- Mahrán, O. M. (2009): *Sarcocystis* infection in sheep and goats slaughtered in Shalatin Abattoir, Red Sea Governorate, Egypt. *Assiut Vet. Med. J.*, 55(121): 341- 355.
- Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, and Woese CR (1997). The RDP (Ribosomal Database Project). *Nucleic Acids Research*, 25(1): 109–111. DOI: <https://doi.org/10.1093/nar/25.1.109>
- Martínez-Navalón, Anastasio-Giner, Cano-Fructuoso, Sanchez-Martínez, Llopis-Morant, et al. (2012). Short communication. *Sarcocystis* infection: a major cause of carcass condemnation in adult sheep in Spain. *Spanish Journal of Agricultural Research*, 10, 388-392.
- Metwally DM, Al-Damigh MA, Al-Turaiki IM and El-Khadragy MF (2019). Molecular characterization of *Sarcocystis* species isolated from sheep and goats in Riyadh, Saudi Arabia. *Animals*, 9(5): 256. DOI: <https://doi.org/10.3390/ani9050256>
- Mohamed, M.; Fatma, M.; Hiekal, A.; Samia, M.; Marwa, M. and Radwan, M. (2016). Characterization of *Sarcocystis* Species Based on Traditional and Molecular Methods in imported Frozen Buffalo Meat in Egypt. *Alexandria J. Vet. Scie*, 51: 155-161.
- Neefs JM, Van de Peer Y, Ruk PDE, Goris A, and Wachter RDE (1991). Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Research*, 19: 1987–2015.

- Ortega-Mora, L. M., Gottstein, B., Conraths, F. J., Buxton, D. Ortega-Mora, L. M., Gottstein, B., Conraths, F. J. & Buxton, D. (2007). Protozoal abortion in farm ruminants: Guidelines for diagnosis Protozoal abortion in farm ruminants: guidelines for diagnosis and control. Wallingford: CABI.
- Şaki, C.E.; Deger, S. and Ozer, E. (2010). Sarcosporidiosis in Turkey. *Yüzüncü Yıl-niversitesi Veteriner Fakültesi Dergisi.*, 21: 129-134.
- Singh, K. P., Agrawal, M. C. and Shah, H. L. (1990): *Vet. Parasitol*, 36: 153-155.
- Wadajkar, S. V., Shastri, U. V. and Narladkar, B. W., (1994): *J.Vet. Parasitol*, 8: 43-46.
- Yang Z, Zuo Y, Yao Y, Chen X, Yang G, and Zhang Y (2001). Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. *Molecular and Biochemical Parasitology*, 115(2): 283–288. DOI: [https://www.doi.org/10.1016/s0166-6851\(01\)00283-3](https://www.doi.org/10.1016/s0166-6851(01)00283-3)

## التعرف على أنواع الساركوسيسيت "Macrocytis" بالطريقة المرئية والجزئية في الأغنام والماعز في مجزرة السليمانية.

شاهين محمد سلام<sup>1</sup> و بهزاد حمدة صالح مصطفى<sup>2</sup>  
قسم علم الحيوان - كلية هندسة العلوم الزراعية - جامعة السليمانية

*Sarcocystis* هو طفيلي يعيش داخل الخلايا (أجباري) يمكن أن يصب الحيوانات والانسان، وتعتبر الأغنام والماعز هما المضيفين الوسيطين لأنواع الساركوسيسيت. أثبتت هذه الدراسة بان الاصابات لها ارتباط معنوي بالجنس والموسم وأنسجة الاعضاء (المرىء والقولون والحجاب الحاجز) ، كما اظهرت النتائج بأن اعلى نسبة اصابة كانت في 5.5% الصيف، 4.7% في الخريف، 4.4% في الفصل الشتاء لتصل الى الحد الأدنى خلال فصل الربيع 4%. أما في الماعز لوحظ اعلى نسبة للاصابة 3.8% في الصيف، 3.6% في الخريف والشتاء لتصل إلى الحد الأدنى في الربيع 2.9%. تم فحص 194897 رأس من الاغنام ( 129635 ذكور و 65262 أنثى) وكانت وجود إصابة 9062 من الاغنام ( 4.7%) منها 6128 ( 67.6%) ذكور و 2934 ( 32.4%) أنثى. تم فحص 27720 ماعز من بينها 17809 ذكور و 9911 أنثى كشفت عن إصابة 969 ( 3.5%) ماعز منها 491 ذكور ( 50.7%) و 478 ( 49.3%) أنثى. أظهرت اعلى معدل للاصابة ب *Macrocytis* في الحجاب الحاجز في ذكور كل من الاغنام 84.4% والماعز 67.3%. واما في اناث الاغنام سجلت اعلى نسبة اصابة في القلب 55%. بينما في اناث الماعز سجلت اعلى نسبة في المرىء 58.5%. هذه العلاقة اعلاه يظهر أهمية كبيرة جدا بين هذه القيمة وفقا لاختبار Chi-square بمستوى  $P < 0.001$ . نتائج الدراسة الجزئية والهيكلية لاصابات بالساركوسيسيت الاغنام والماعز في السليمانية كردستان العراق. حيث تم تضخيم الجين 18S rRNA الجزئي في جميع العينات المختبرة واظهرت حجم PCR amplicon المتوقع من 600 BP و اظهرت هذه النتيجة وفقا للباندا ايجابية وكانت الانواع الساركوسيسيت المعزولة الأكثر ارتباطا بها *S. gigantea*, *S. moulei*, and *S. medusiformis* وربما تعتبرها سلالات شقيقة العدوى الطفرة من الجينات قد يحدث بين الأغنام والماعز و بالتالي فان خصوصية المضيف من العديد أنواع الساركوسيسيت مشكوك فيها